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FORM PTO-1390 (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK, OFFICE ATTORNEY'S DOCKET TRANSMITTAL LETTER TO THE UNITED STATES PC10433A PATENT AND TRADEMARK OFFICE U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) DESIGNATED/ELECTED OFFICE (DO/EO/US) filed herewith via EXPRESS MAIL LABEL CONCERNING A FILING UNDER 35 U.S.C. 371 EE 645346652 US INTERNATIONAL APPLICATION NO. INTERNATIONAL FILING DATE PRIORITY DATE CLAIMED PCT/US00/04733 February 25, 2000 February 26, 1999 TITLE OF INVENTION METHOD FOR THE PURIFICATION, RECOVERY, AND SPORULATION OF CYSTS AND OOCYSTS APPLICANT(S) FOR DO/EO/US Harold N. CONKLE; Joseph E. SCHULTZ; Scott J. BLONIGEN; Fred H. WEBER; David R. KILANOWSKI; Bruce MONZYK; Timothy M. WERNER, Chad M. CUCKSEY; Hamish A.I. McARTHUR; and Ted L. TEWKSBURY Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: This is the FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. This is the SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 4. A copy of the International Application as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (required only if not transmitted by the International Bureau). b has been transmitted by the International Bureau. is not required, as the application was filed in the United States Receiving Office (RO/US). A translation of the International Application into English (35 U.S.C. 371(c)(2)). Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) are transmitted herewith (required only if not transmitted by the International Bureau). a Ь have been transmitted by the International Bureau. have not been made; however, the time limit for making such amendments has NOT expired. have not been made and will not be made. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C 371(c)(3)) 9. An oath or declaration of the inventor(s) (35 U S.C. 371(c)(4)) In Unexecuted Form Only 10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S.C. 371(c)(5)) Items 11. To 16. Below concern other documents(s) or information included: 11. An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98 with Form A820/1449; copies of 8 references. 12. An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included. 13. \times A FIRST preliminary amendment A SECOND or SUBSEQUENT preliminary amendment. П 14. A substitute specification. 15. A change of power of attorney and/or address letter. Other items or information: 16. Copy of International Search Report Copy of Invitation to Pay Additional Fees -- for Multiple Inventions Applicants "Response to Pay Additional Fees" for Multiple Inventions Certificate of Mailing via Express Mail EE 645346652 US Return Receipt Postcard

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I hereby addressed	certify that this correspondence is being deposited with the United States d to: Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20	Postal Ser 0231 on thi	vice as Express Mail in an envelope s 30 day of November 2000.
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FILIN	G DATE: November 30, 2000	:	Assigned
TITLE	E: Method for the Purification, Recovery, and Sporulation of Cysts and Oocysts.	:	
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Sir:

Preliminary Amendment

At page 1 of the Specification, on blank line No. 2 (directly beneath the Title Sentence), please insert the following paragraph.

-- The present application is the United States national stage (35 USC 371) of International Application PCT/US00/04733, internationally filed on February 25, 2000. The present national application claims priority under 35 USC 119(e) to U.S. Provisional Application 60/122,160, filed February 26, 1999, the complete text, claims and figures of which are incorporated by reference herein, as if fully set forth. --

Respectfully submitted,
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E. U.A.N.

11-30-00

Date:

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Method For The Purification, Recovery, And Sporulation of Cysts And Oocysts

Background of the Invention

This invention relates generally to methods for the purification, recovery, and sporulation of encysted protozoa for use in the production of vaccines. Protozoa are pathogens known to attack the gastrointestinal tract of the host. In a situation where the host has a weak or suppressed immune system, such as very young, very old, and immuno-compromised hosts, infection may be fatal. Such a loss can translate into an economic loss as well. Protozoa are present in the environment in a relatively stable cyst form, also termed an occyst when the protozoa is a sporozoa. Upon ingestion into the host, the encysted protozoa responds to the conditions of the gastrointestinal tract and infects the host.

In order to prevent or alleviate the problem of these diseases, non-in-ovo vaccines have been developed. These vaccines typically expose the host to a low level of the protozoa in order to develop immunity in the host without causing disease. Such vaccines include a vaccine against avian coccidiosis caused from any one of a number of species of coccidia (U.S. Patent No. 5,055,292), a vaccine for cats against toxoplasma (U.S. Patent No. 5,045, 313), and vaccines for pigs and other ruminants (U.S. Patent No. 4,808,404). These vaccines are only partially effective because they must be given by gavage or applied to the water or the food of the animal. Gavage is ineffective because it requires manual vaccine introduction. Also, many chicks die due to handling. Adding the vaccine to the food or water is also ineffective as young chicks eat little directly after hatching; therefore, disease control is delayed. The result is less rapid weight gain and a longer time to reach market weight.

In order to produce the vaccine, a supply of adequately purified encysted protozoa must be obtained. Current separation and recovery techniques employ corrosive, hazardous, toxic materials. Measures must be undertaken to compensate for these materials such as extensive washing operations to remove these materials which results in low product yield. The typical method of obtaining the encysted protozoa includes obtaining a source of encysted protozoa, such as the intestines or feces of infected animals. The intestinal matter or fecal matter needs to be separated from the encysted protozoa, and a flotation method is used. Heavy media flotation or sedimentation processes have been used where the heavy media is sodium chloride (U.S. Patent No. 4,863,731) or sucrose (U.S. Patent No. 5,068,104). The heavy media is used in an aqueous mixture. Unfortunately, the use of aqueous mixtures of the heavy media can have adverse effects. For example, aqueous mixtures of sodium chloride can lead to severe equipment corrosion.

Before the encysted sporozoa can become infective, the separated oocysts must be sporulated by mild oxidation. Mild oxidation is accomplished by either placing the suspension on a shaking table or bubbling air through the suspension of the oocysts. Potassium dichromate is typically employed to suppress any unwanted microbial growth during sporulation. But, potassium

dichromate is a hazardous material. Its use creates handling and disposal problems. Its removal results in reduced yield and increased costs.

A final bleaching step using sodium hypochlorite is used to eliminate any remaining organic material and undesired microorganisms. The bleached encysted protozoa must be washed to reduce the residual bleaching agent concentration to an acceptable level. Washing is accomplished by a series of dilutions and oocyst recovery operations to reduce the bleaching agent levels while producing an occyst concentrate. The final encysted protozoa concentrate is made into a vaccine under sterile conditions. Each vaccine may include multiple species of protozoa to provide the greatest amount of protection possible.

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A need exists for a more efficient vaccination method. Such a vaccination method would employ a live vaccine dosed at a level sufficient to generate an immuno-response to build immunity but low enough not to cause acute symptoms. For example, an in-ovo vaccine against avian coccidiosis can shorten treatment times and allow a more uniform dosage. This method could also be used to collect and produce human vaccines for protection against protozoa including Cryptosporidium and Giardia lamblia. The method would employ a non-corrosive dense media flotation process or a gas flotation process that eliminates the need for salt entirely. This improved method would not use a hazardous biocide such as potassium dichromate. Such an alternative biocide or oxidant could also double as a bleaching agent, eliminating another step in the process and another hazardous compound.

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Summary of the Invention

The present invention is directed to a method for the purification and recovery of encysted protozoa, including separating the encysted protozoa from a suspension containing the encysted protozoa by either a salt flotation process where the salt is salts such as sodium sulfate. magnesium sulfate, magnesium chloride, calcium chloride, or mixtures thereof, or a gas flotation process.

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In one embodiment, the method of separating the encysted protozoa is accomplished by a salt flotation process and the salt is sodium sulphate. In this embodiment, the method includes preparing an admixture comprising the encysted protozoa and the sodium sulphate, centrifuging the slurry and recovering a supernatant therefrom, forming a dilution of the supernatant and centrifuging the dilution, and recovering the concentrate from the centrifuged dilution. This embodiment may also include homogenizing the admixture by high intensity homogenization. The sodium sulphate is present in the admixture in an amount from about 3 to about 30 weight percent. Further, the specific gravity of the dilution may be less than the specific gravity of the encysted protozoa. The concentrate comprises from about 1x10⁴ to about 1.5x10⁶ encysted protozoa/ml.

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In another embodiment, separation of the encysted protozoa is accomplished by the gas flotation process. The gas flotation process includes adjusting the suspension to a pH sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa, conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent

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compound to selectively coat particles in the suspension and a sufficient amount of a heteropolar compound to produce a stable froth, passing the conditioned suspension through at least one gas flotation cell, and recovering the encysted protozoa from the gas flotation cell. Suitable gases include air. When the gas is air, the pH can be adjusted to about 2.5 to about 3.5. The surface active agent compounds include a sodium salt of long-chain alkyl hydrogen sulfate, a quaternary ammonium compound, a blend of a fatty ammonium acetate and 2-ethylhexanol, an ester/amide compound, an alkyloxy polyethylenoxyethanol, and mixtures thereof. The heteropolar compounds include amyl alcohols, butyl alcohols, terpinols, cresols, and mixtures thereof. Suitable gas flow rates for the gas flotation cell range from about 0.25 to about 1.1 volumes of gas per volume of suspension per minute. In a preferred embodiment, the gas flotation cell comprises at least two serial gas flotation units, and these units can have different gas flow rates.

The present invention is also directed to method for the sporulation of oocysts which includes forming an aqueous suspension of the oocysts with water and hydrogen peroxide, wherein the hydrogen peroxide is present in an amount sufficient to eliminate unwanted microbiological growth, and aerating the aqueous suspension to sporulate the oocysts. The aqueous suspension may be aerated for a time period greater than about 40 hours under conditions such that the aqueous suspension during aeration has a dissolved oxygen level greater than about 80% of the saturation level at a temperature of about 22°C to about 32°C and may have an agitation level sufficient to adequately suspend all the solids. In this embodiment, the aqueous suspension can include an oocyst concentration of about 10⁴ to about 10⁶ oocysts/ml and a hydrogen peroxide.

In a further embodiment, the present invention covers a method for the purification, recovery, and sporulation of oocysts including separating the oocysts from a first suspension comprising the oocysts, and sporulating the separated oocysts by the methods of the present invention. Suitable oocysts are Eimeria maxima, Eimeria mittis, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria necatrix, Eimeria praecox, and mixtures thereof. In one embodiment, separation of the oocysts is accomplished by a sodium sulfate flotation process which includes preparing an admixture comprising the oocysts and the sodium sulphate, centrifuging the slurry and recovering a supernatant therefrom, forming a dilution of the supernatant and centrifuging the dilution, and recovering the concentrate from the centrifuged dilution.

In another embodiment, separation the oocysts is accomplished by a gas flotation process which includes adjusting the first suspension to a pH sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa, conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent compound to selectively coat particles in the suspension and a sufficient amount of a heteropolar compound to produce a stable froth, passing the conditioned suspension through at least one gas flotation cell, and recovering the encysted protozoa from the gas flotation cell. This embodiment may further include adding a bleaching agent to the sporulated oocysts in an amount sufficient to inactivate residual microorganisms and eliminate residual organic matter, and bleaching the sporulated oocysts. In

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another embodiment, the bleaching is conducted concurrently with the sporulation, and the bleaching agent is hydrogen peroxide. In yet another embodiment, the bleaching agent is sodium hypochlorite present in an amount from about 5,000 to about 10,000 parts per million free available chlorine, ozone present in an amount up to about 3% in air, and combinations thereof.

This method may further include washing the bleached oocysts by cross-flow membrane filtration to decrease the residual bleaching agent concentration to an acceptable level. In yet another embodiment, the bleaching agent is sodium hypochlorite present after washing in a concentration sufficient to suppress residual microbial growth, and the bleached and washed oocyst suspension has a concentration from about 1 x 10° to about 2.5 x 10° oocysts/ml, a maximum solids size of less than about 200 microns, and sodium sulphate present in an amount sufficiently low to not interfere with the viability and infectivity of the cysts or oocysts, preferably less that about 3 percent, more preferably less than about 0.9 percent. This method may further include concentrating the bleached and washed oocysts into a sterile concentrate, combining sterile concentrates of one or more species of oocysts into a combined concentrate, and packaging the combined concentrate under sterile conditions.

Brief Description Of The Drawings

Fig. 1A is a flow chart illustrating embodiments of a first portion method of the present invention;

Fig. 1B is a flow chart illustrating embodiments of a second portion method of the present invention;

Fig. 2 is a plot showing oocyst concentration verses sodium sulfate concentration; and

Fig. 3 is a plot showing sporulation percent verses time after cyst harvest.

Detailed Description Of The Invention

The present method is directed to producing a concentrate of cysts or sporulated oocysts to be utilized in a vaccine. Specifically, the method is directed to producing a concentrate of sporulated avian coccidial oocysts to be used to produce an in-ovo vaccine against coccidiosis. The production of a suitable and acceptable vaccine requires a multi-step treatment process constituting a plurality of unit processes. As shown in Figs. 1A and 1B, the vaccine production process can include taking a suspension of encysted protozoa, homogenizing the suspension, separating the encysted protozoa from the suspension by either salt flotation or gas flotation, sporulating the encysted protozoa if required, bleaching the sporulated encysted protozoa, washing the bleached product, concentrating the encysted protozoa, combining the concentrates from multiple encysted protozoa, and sterile filling to produce a vaccine. These steps are described in further detail below.

Encysted protozoa, which includes cysts and oocysts, may be obtained from various sources including purified suspensions, intestinal linings, and fecal suspensions. Especially when the encysted protozoa are obtained from feces, the suspensions or slurries can include significant

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amounts of undesirable suspended solids. For example, the suspensions can include from about 1 up to about 20 weight percent solids or feces. Preferably, the suspension includes from about 5 to about 20 weight percent solids when salt flotation is used and about 1 weight percent when gas flotation is used. In order to liberate the cysts from the associated organics in the suspension, the suspension may be subjected to a homogenization step. The suspension may be homogenized for a time period from about greater than 0 to about 5 minutes, preferably 1 minute depending upon the intensity of the homogenization. The homogenization conditions should be sufficiently intense to liberate the cysts and oocysts but gentle enough to prevent their destruction.

The encysted protozoa need to be separated from this first suspension, preferably to achieve at least about 70% encysted protozoa recovery and at least about 80% solids rejection. Typically, this results in an encysted protozoa concentration of about 1x10⁴ to about 1.5x10⁶ encysted protozoa/ml. Separation can be achieved by either a salt flotation process or a gas flotation process.

When a salt flotation process is used, an admixture of the unpurified encysted protozoa and the salt is prepared. Suitable salt solutions include dense solutions of water soluble salts including chlorides, sulfates, phosphates, nitrates, and acetates of ammonium, sodium, potassium, calcium, magnesium, and zinc. Suitable highly hydrogen-bonded organics including urea and the salts of guanidiene. Preferably, the salts include sodium sulfate, sodium chloride, magnesium sulfate, magnesium chloride, calcium chloride, and mixtures thereof. More preferably, the salt is sodium sulfate. These salts provide the benefit of being significantly less corrosive to the process equipment. The salt is added in an amount sufficient to produce a difference between the specific gravities of the encysted protozoa and the admixture. Preferably, the specific gravity of the encysted protozoa will be less than the specific gravity of the admixture. The salt is present in an amount from about 3 to about 30 weight percent, preferably about 14 to about 20 weight percent, more preferably about 20 weight percent.

The admixture is then subject to centrifugation and the supernatant and concentrate are collected. Depending upon the admixture conditions, the encysted protozoa can be contained in the supernatant or the concentrate. The admixture is centrifuged at about 3000 to about 15,000g, preferably 12,000g for a period of time necessary to adequately separate the encysted protozoa, typically up to about 10 minutes. For a 20% sodium sulfate solution, the encysted protozoa will be contained in the supernatant; therefore, the supernatant is collected and the concentrate or pellet is directed to waste. As shown in Fig. 2, a sodium sulfate concentration of 20% provides for a high concentration of encysted protozoa (oocysts in this case) in the supernatant (product) and only a small amount of encysted protozoa in the pellet (waste). A dilution is formed by the addition of water to the recovered supernatant. The supernatant is diluted sufficiently so that the specific gravity of the encysted protozoa is more than the specific gravity of the dilution. Therefore, the encysted protozoa will tend to settle in the dilution. For a sodium sulfate concentration of 20%, the ratio of the weight of the dilution to the weight of the supernatant is from about 4 to about 8.

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The dilution is then centrifuged for a second time under comparable centrifugation conditions as in the first centrifugation, such that the residual salt concentration is less than about 10 weight percent, preferably less than about 1 weight percent, and the resulting cyst concentration is about 10⁴ to 10⁶ cysts/ml representing about 80 to about 95 percent recovery. The solid debris rejection rate is preferably about 90 to about 99 percent. In this embodiment, the encysted protozoa are recovered from the concentrate and the supernatant is directed to waste.

In another embodiment, separation of the encysted protozoa is accomplished by a gas flotation process. Preferably the gas is air, although any gas including oxygen and nitrogen could be used. When gas flotation is used, the pH of the suspension is adjusted to a pH sufficient to affect adhesion between the gas bubbles in the suspension and the encysted protozoa by changing the surface chemistry of the encysted protozoa. The pH is typically adjusted to a pH of about 2 to about 9, preferably about 2.5 to 3.5, more preferably about 3.

The pH adjusted suspension is additionally conditioned by the addition of a surface active agent compound, sometimes referred to as a collector compound and a heteropolar compound, sometimes referred to as a frother compound. The surface active agent compound, also termed a selective detergent, wetting agent, or emulsifier, is added in an amount sufficient to promote contact between selected solids and the gas bubbles by forming a thin coating over the particles to be floated, rendering these particles hydrophobic, while not coating other particles. Therefore, through selection of the surface active agent compound, the particles to be floated can be determined. Therefore, the encysted protozoa could be collected in a gas flotation process in which they are floated or in a reverse flotation process in which the solids to be removed are floated. Suitable surface active agents include a sodium salt of long-chain alkyl hydrogen sulfate, a quaternary ammonium compound, a blend of a fatty ammonium acetate and 2-ethylhexanol, an ester/amide compound, an alkyloxy polyethylenoxyethanol, and mixtures thereof. Preferably, the surface active agent compound is sodium lauryl sulfate. The surface active agent should be present in an amount sufficient to coat the particles to be floated. The surface active agent is present in an amount from about 0.5 to 2 lb per ton of solids in the conditioned suspension, preferably 0.5 lb per ton of solids. Alternatively, the surface active agent compound can be about 0.5 lb dodecyl amine per ton of solids which has been acidified with hydrochloric acid until a neutral pH is obtained, or about 0.5 lb potassium salt of oleic acid per ton of solids.

The heteropolar compounds are selected for their ability to change the surface tension of the water and produce stable froths. Typically, these heteropolar compounds contain one or more hydrocarbon groups attached to one polar group, with the hydrocarbon radical having upwards of 5 or 6 carbon atoms. Suitable heteropolar compounds include amyl and butyl alcohols, terpinols, cresols, and mixtures thereof. Preferably, the heteropolar compound is methyl isobutyl carbinol (MIBC) also referred to as methyl amyl alcohol and most accurately referred to as 4-methylpentanol,-2. The heteropolar compound is present in an amount sufficient to produce a stable froth. Preferably, the heteropolar compound is present in an amount up to about 2.0 lb per ton of

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solids, more preferably from about 0.5 to 2.0 lb per ton of solids, most preferably about 0.5 lb per ton of solids.

The conditioned suspension is passed through a gas flotation cell and the encysted protozoa are recovered. Depending upon the pH, surface active agent compound, and heteropolar compound selected, the encysted protozoa may be floated or retained in suspension. Preferably, the encysted protozoa are floated. The gas flotation process is conducted for a period of time and at an air flow rate sufficient to recover about 20 to 100 percent, typically about 85 percent of the encysted protozoa while rejecting about 20 to 90 percent, typically 70 percent of the solid debris. The gas flotation process is conducted for a period of time of about 3 minutes to greater than about 10 minutes, preferably about 10 minutes. The gas flow rate in the air flotation cell is about 0.25 to about 1.1 volumes of gas per volume of solution per minute ("vvm"), preferably about 0.25 to about 0.83 vvm, more preferably about 0.25 to about 0.75 vvm. The gas flotation cell may include a plurality of serial flotation units. Preferably, the gas flotation cell includes at least two serial flotation units. Preferably, the flotation units are gas flotation columns. The gas flow rate in the serial flotation units may be the same or different. Preferably the gas flows are different, for example about 0.47 vvm for a 32 inch high, 2 inch diameter column.

When the encysted protozoa are oocysts or encysted sporozoa, the oocysts are sporulated. As is shown in Fig. 3, sporulation should occur as soon after harvesting the oocysts as possible, preferably, the oocysts should be sporulated within 3 days after harvest. An aqueous suspension of the oocysts and an oxidant or biocide is prepared, and the aqueous suspension is aerated for a period of time sufficient to sporulate the oocysts. If a salt flotation process was used to separate the oocysts, then the salt concentration during sporulation should be less than about 10 weight percent, preferably less than about 2 weight percent, more preferably less than about 1 weight percent. Preferably, the cyst concentration during sporulation is about 104 to about 106 oocysts/ml, more preferably about 105 oocysts/ml. The oocysts should be sporulated within about 1 to 24 hours after recovery of the purified cyst concentrate. The pH of the aqueous suspension should be about 5.0 to 7.0, preferably about 5.2 to 6.8. The temperature of the aqueous suspension in one embodiment is about 20 to about 33 °C, preferably about 22 to about 32°C, more preferably about 25 to about 29°C, most preferably about 25°C. The aeration rate in one embodiment is about 0.1 to about 10 vvm, preferably about 0.1 to about 2.0 vvm. Overall, the agitation level should be sufficient to fully suspend all solids during sporulation but not enough to destroy the oocysts. This may occur through aeration, shaking, stirring, and combinations thereof. When stirring is used, for example, the stirring should be sufficient to keep the suspended solids in suspension but not enough to destroy the cysts or oocysts. Suitable stirring in a 6 inch diameter vessel can occur at about 121 to 204 rpm, preferably about 197 to 200 rpm, more preferably about 200 rpm. The dissolved oxygen content should be maintained at a level sufficient to permit the oocysts to sporulate, preferably about 80% of the saturation concentration at the given temperature. The time period for sporulation can be

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up to about 72 hours, preferably between about 40 and about 72 hours, more preferably about 48 to about 50 hours.

The oxidant is added in a sufficient amount to inactivate the undesirable microbial growth in the aqueous suspension. Suitable oxidants, or biocides, include hydrogen peroxide, ozone, potassium dichromate, chlorine, and combinations thereof. In one embodiment, the oxidant is potassium dichromate and is present in an amount of about 2.5 v/w percent. In a preferred embodiment, the oxidant is hydrogen peroxide present in an amount from about 1,000 to about 20,000 mg/l, preferably about 5,000 mg/l. Hydrogen peroxide provides the benefit of easier and less expensive handling, and the generation of a hazardous waste by-product from this process is alleviated.

Aeration can be accomplished by either shaking on a shaker table or air sparging in a sporulation tank (e.g. fermentation tank). Preferably, aeration is accomplished in an air sparging tank because the mass transfer of air is greater, thus reducing the time for sporulation and the required size of the equipment.

Following sporulation, if necessary, the oocysts may be washed to reduce the residual oxidant concentration to an acceptable level. Hydrogen peroxide provides the benefit of eliminating the need for this step. Washing may be accomplished by serial washings, preferably, washing is accomplished by membrane filtration, more preferably by diafiltration. In the case of membrane filtration, the membrane pore size is selected to allow passage of solutes through the membrane while restricting the passage of the oocysts from one side of the membrane to the other. In one embodiment, washing is conducted with water at a transmembrane pressure of about up to about 30 psi, preferably about 20 to about 25 psi, a crossflow velocity of up to about 10 m/s, preferably about 2 m/s, and a flux through the membrane of up to about 10 l/min/m², preferably about 3 l/min/m².

The oocysts may be bleached to inactivate residual microorganisms and to eliminate residual organic matter. First, a sufficient amount of a bleaching agent is added, and then the oocysts are bleached or contacted with the bleaching agent for a sufficient period of time. The oocysts may be bleached for a period of up to 1.5 hours. Suitable bleaching agents include sodium hypochlorite, hydrogen peroxide, ozone, and mixtures thereof. The bleaching agent can be initially present in an amount of about 2,000 to about 20,000 mg/l, preferably about 8000 to about 10,000 mg/l, more preferably, 8000 mg/l. The bleaching agent should not be present in an amount sufficient to cause corrosion in the process equipment. When hydrogen peroxide is used for sporulation, bleaching and sporulation may be conducted concurrently. Following bleaching, the bleached suspension is washed, if necessary, to reduce the residual oxidant concentration to an acceptable level. When sodium hypochlorite is the bleaching agent, the acceptable level is less than about 1 mg/l. Washing can be accomplished by either serial washing or diafiltration.

The bleached suspension can be concentrated into a sterile concentration having a concentration high enough for efficient and effective handling. For example, the final concentrated encysted protozoa suspension can include a maximum solids size of less that about 200 microns,

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preferably less than about 25 microns, a salt content of less than about 0.9 percent, a free residual chlorine concentration of less than about 1 mg/l but sufficient to keep residual microbial growth suppressed, and a cyst concentration of about 1×10^6 to 2.5×10^6 cysts/ml.

When numerous encysted protozoa or different species of one genus of encysted protozoa are to be used in a single vaccine, the sterile concentrates from the different species are combined into a single sterile concentrate. For example, the encysted protozoa can be avian coccidial oocysts including Eimeria maxima, Eimeria mitis, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria necatrix, Eimeria praecox, and mixtures thereof including multiple strains of each. Finally the combined concentrates are subject to filling and packaging under sterile conditions, and a vaccine is produced.

Although preferred embodiments of the present invention have been described, it is understood that the present method can be used to produce other vaccines for numerous types of animal and for humans. This method could, in fact, be used to immunize humans against typical waterborne protozoa such as *Cryptosporidium* and *Giardia lamblia*. Moreover, even though the entire recovery process is described, unit processes thereof may be used independently or inserted into other recovery, monitoring, or treatment schemes without departing from the scope and spirit of the present invention.

The Claims

What is claimed is:

1. A method for the purification and recovery of encysted protozoa, comprising separating the encysted protozoa from a suspension comprising the encysted protozoa by a salt flotation process wherein the salt comprises sulfates, phosphates, nitrates, acetates of ammonium, sodium, potassium, calcium, magnesium, or zinc, hydrogen-bonded organics, the salts of guanidiene, or mixtures thereof, or a gas flotation process.

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2. The method of claim 1, wherein separating the encysted protozoa is accomplished by a sodium sulphate flotation process which comprises:

preparing an admixture comprising the encysted protozoa and the sodium sulphate; centrifuging the slurry and recovering a supernatant therefrom; forming a dilution of the supernatant and centrifuging the dilution; and

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The mixture of claim 2, further comprising: homogenizing the admixture by high intensity homogenization.

recovering the concentrate from the centrifuged dilution.

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4. The method of claim 2, wherein the sodium sulphate is present in the admixture in an amount from about 3 to about 30 weight percent.

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- The method of claim 2, wherein the specific gravity of the dilution is less than the specific gravity of the encysted protozoa.
 - The method of claim 2, wherein the concentrate comprises from about 1x10⁴ to about 1.5x10⁶ encysted protozoa/ml.
- 7. The method of claim 1, wherein separating the encysted protozoa is accomplished by the gas flotation process which comprises:

adjusting the suspension to a pH sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa;

conditioning the pH adjusted suspension by adding a sufficient amount of a surface active

35 agent compound to selectively coat particles in the suspension and a sufficient amount of a
heteropolar compound to produce a stable froth;

passing the conditioned suspension through at least one gas flotation cell; and recovering the encysted protozoa from the gas flotation cell.

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- 8. The method of claim 7, wherein the gas is air.
- 9. The method of claim 8, wherein the suspension pH is about 2.5 to about 3.5.
- 5 10. The method of claim 7, wherein the surface active agent compound comprises a sodium salt of long-chain alkyl hydrogen sulfate, a quaternary ammonium compound, a blend of a fatty ammonium acetate and 2-ethylhexanol, an ester/amide compound, an alkyloxy polyethylenoxyethanol, or mixtures thereof.
- 10 11. The method of claim 7, wherein the heteropolar compound comprises amyl alcohols, butyl alcohols, terpinols, cresols, or mixtures thereof.
 - 12. The method of claim 7, wherein the gas flotation cell has a gas rate from about 0.25 to about 1.1 volumes of gas per volume of suspension per minute.
 - 13. The method of claim 7 wherein the gas flotation cell comprises at least two serial gas flotation units.
 - 14. The method of claim 13, wherein the at least two units comprise different gas flow rates.
 - 15. A method for the sporulation of oocysts, comprising:

forming an aqueous suspension of the oocysts with water and hydrogen peroxide, wherein the hydrogen peroxide is present in an amount sufficient to eliminate unwanted microbiological growth; and

- aerating the aqueous suspension to sporulate the oocysts.
- 16. The method of claim 15, wherein the aqueous suspension is aerated for a time period greater than about 40 hours such that the aqueous suspension during aeration has a dissolved oxygen level greater than about 80% of the saturation level at a temperature of about 22°C to about 32°C and with an agitation level sufficient to adequately suspend all the solids.
- 17. The method of claim 15 wherein the aqueous suspension comprises an oocyst concentration of about 10⁴ to about 10⁶ oocysts/ml and an initial hydrogen peroxide concentration of about 1,000 to about 20,000 mg/l.
- 18. A method for the purification, recovery, and sporulation of oocysts, comprising: separating the oocysts from a first suspension comprising the oocysts; and sporulating the separated oocysts by the method of claim 14.

- 19. The method of claim 18 wherein the oocysts are Eimeria maxima, Eimeria mitis, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria necatrix, Eimeria praecox, or combinations thereof.
- 20. The method of claim 18 wherein separating the oocysts is accomplished by a sodium sulfate flotation process which comprises:

preparing an admixture comprising the oocysts and the sodium sulphate; centrifuging the slurry and recovering a supernatant therefrom; forming a dilution of the supernatant and centrifuging the dilution; and recovering the concentrate from the centrifuged dilution.

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21. The method of claim 18 wherein separating the oocysts is accomplished by a gas flotation process which comprises:

adjusting the first suspension to a pH of sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa;

conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent compound to selectively coat particles in the suspension and a sufficient amount of a heteropolar compound to produce a stable froth;

passing the conditioned suspension through at least one gas flotation cell; and recovering the encysted protozoa from the gas flotation cell.

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22. The method of claim 21, further comprising:

adding a bleaching agent to the sporulated oocysts in an amount sufficient to inactivate residual microorganisms and eliminate residual organic matter; and bleaching the sporulated oocysts.

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- 23. The method of claim 22, wherein the bleaching is conducted concurrently with the sporulation, and the bleaching agent is hydrogen peroxide.
- 24. The method of claim 22, wherein the bleaching agent is sodium hypochlorite present in an amount from about 5,000 to about 10,000 parts per million free available chlorine, ozone present in an amount up to about 3% in air, or combinations thereof.
 - 25. The method of claim 22, further comprising washing the bleached oocysts by cross-flow membrane filtration to decrease the residual bleaching agent concentration to an acceptable level.

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26. The method of claim 25, wherein the bleaching agent is sodium hypochlorite present after washing in a concentration sufficient to suppress residual microbial growth, and the bleached and washed occyst suspension has a concentration from about 1 x 10° to about 2.5 x 10° occysts/ml, a

maximum solids size of less than about 200 microns, and a sodium sulphate content of less than about 0.9 percent.

27. The method of claim 26 further comprising:

concentrating the bleached and washed oocysts into a sterile concentrate; combining sterile concentrates of one or more species of oocysts into a combined concentrate; and

packaging the combined concentrate under sterile conditions.

Method For The Purification, Recovery, And Sporulation Of Cysts And Oocysts ABSTRACT

A vaccine for in ovo vaccination against avian coccidiosis produced by a method

including obtaining the coccidial occysts from a fecal suspension, homogenizing the fecal suspension, separating the occysts from the fecal debris by either salt flotation using sodium sulfate or gas flotation using air, sporulating the occysts using hydrogen peroxide and air sparging, bleaching the sporulated occysts, washing the bleached occysts, concentrating the sterile washed occysts and combining the concentrates of various species of coccidial occysts, and producing a vaccine. The method in whole or in part can be applied to other kinds of encysted protozoa to produce vaccines for various types of animals.

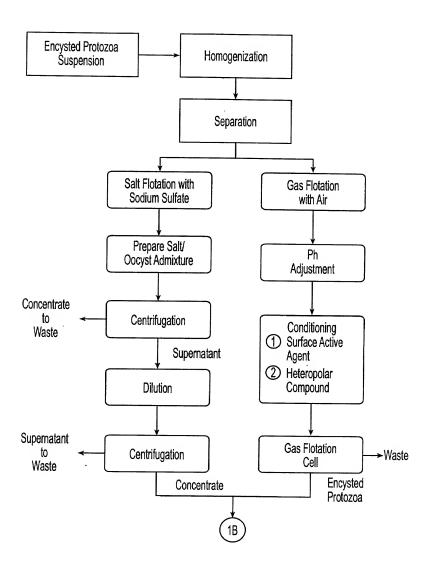
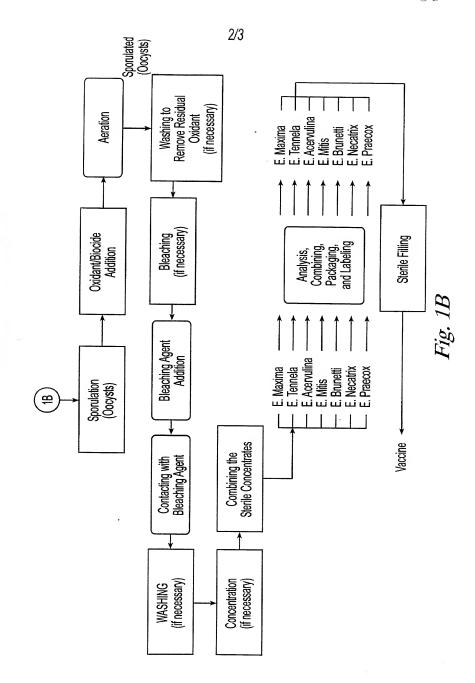


Fig. 1A



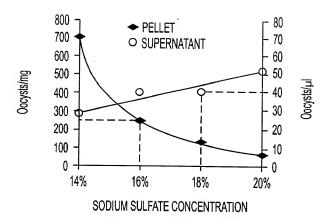


Fig. 2

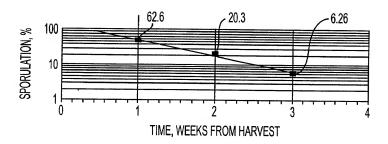


Fig. 3

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

Declaration submitted with Initial Filing

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Declaration Submitted after Initial
Filing (surcharge 37 CFR 1.16 (e))
required)

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Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLE	TE IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

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		(Title of the Invention)			
the specification of which is attached hereto					
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DECLARATION

Name of Additional Joint	t Inventor, if any	/: [] A petit	ion has be	en filed for thi	s unsigned inve	ntor	
Given Name (f	irst and middle [i	f any])				Family Name	e or Surname	
Ted L.				Tewksbu	iry			
Inventor's Signature							Date	
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Post Office Address	1309 Deerlick	Drive	740			L		
Post Office Address			\sim \sim					W. C
City	Columbus	State	ОН	Zip	43228	Country	US	
Name of Additional Joint	Inventor, if any	: [] A petit	on has be	en filed for the	s unsigned invei	ntor	
Given Name (f	irst and middle [i	f any])				Family Name	e or Surname	
Inventor's Signature							Date	
Residence: City			State	T	Country		Citizenship	
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Name of Additional Joint	Inventor, if any	: [] A petit	on has be	en filed for thi	s unsigned inver	ntor	
	Inventor, if any] A petit	on has be	en filed for thi		ntor e or Surname	
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Given Name (f Inventor's Signature Residence: City				on has be			or Surname Date	
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Given Name (f Inventor's Signature Residence: City Post Office Address Post Office Address	urst and middle (i	f any]) State	State	Zip	Country	Family Name	Date Citizenship	
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COPPLIED. OHIOLI

DECLARATION FOR UTILITY OR
DESIGN
PATENT APPLICATION
(37 CFR 1.63)

Declaration submitted with Initial Filing

Declaration
Submitted after Initial
Filing (surcharge
37 CFR 1.16 (e))
required)

Takin and Traceman	KONICE CO DEI ARTIMENTO COMMENCO
Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLE	TE IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

My residence, post office address believe I am the original, first a names are listed below) of the s	ss, and citizenship ar				
		re as stated below next to my na	ime.		
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METHOD FOR THE PURIFICA	ATION, RECOVERY,	, AND SPORULATION OF CYS	TS AND OOCYSTS	3	
		(Title of the Invention)		····	
the specification of which is attached hereto					
OR					
was filed on (MM/DD/YY	YY) 02/25/2000	as PCT I	nternational		
Application Number PCT/U	S00/04733 and w	as amended on		(if applicab	le).
I hereby state that I have review	(MM/I	DD/YYYY)	d an alfantin		
amended by any amendment sp			ed specification, in	cluding the claims	s, as
I acknowledge the duty to disclo	se information which	us material to natentability as d	efined in 37 CFR 1	56	
I hereby claim foreign priority be certificate, or 365(a) of any PC					
America, listed below and have	also identified pelow	, by checking the box, any foreig	gn application for pa	tent or inventor's	
or of any PCT international appl	ication naving a tiling		<u>-</u>		
Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Co YES	py Attached? NO
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Additional foreign application of	numbers are listed on	a supplemental priority data sh	eet PTO/SB/028 at	tached hereto:	
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DECLARATION ---- Utility or Design Patent Application

hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior

U.S. Parent Application Number or PCT Parent Number (MM/DDYYYY) Parent Parent Number (If applicable)	information who	ch is maleri	rnational applicati al to patentability national filing date	as define	d in 37 US	ided by C 156	the first i, which	paragrap became	h of 35 available	USC 1 between	12, I a the fi	cknowledg ling date	e the duty to c of the prior app	lisclose lication
As a named inventor. I hereby appoint the following registered gracultioner(s) to prosecute this application and to transect all ousness in the Patent and Trademark Office connected therewith Customer Number	U.S. Parent A			T Parent						Pa				
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Allen J. Spiegel		Name			Registratio		Ť							
Paul H. Ginsburg	Peter C. Rich	nardson			27,526		A	. Dean	Olson				31,185	
J. Trevor Lumb											- [
James T. Jones 30,561 Robert T. Ronau 36,257											- 1			
Sergeg C. Benson 30,977 B. Timothy Creagan 39,156 Robert F. Sheyka 31,304 Alan L. Koller 37,371 Grover F. Fuller Jr. 31,760 Jolene W. Appleman 35,428 Karen DeBenedictis 32,977 Kristina L. Konstas 37,864 Lorraine B. Ling 35,251 Seth H. Jacobs 32,140 Garth Butterfield 36,997 Martha A. Gammill 31,820 Carl J. Goddard 39,203 Gregory P. Raymer 36,647 Raymond M. Speer 26,810 E. Victor Donahue 35,492 Jennifer A. Kispert 40,049 Roy F. Waldron 42,208 Israel Nissenbaum 27,582 Todd M. Crissey 37,807 Addian G. Looney 41,406 Deborah A. Martin 44,222 A. David Joran 37,858 Jeffrey N. Myers 41,213 Lawrence C. Akers 28,587 Elsa Djuardi 45,963 Gabriel L. Kleiman 40,681 Michelle A. Sherwood 36,271 Donna R. Grossu P-47,248 Arlene K. Musser 37,895 Addifficular legistered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to:											- 1			
Robert F. Sheyka 31,304 Alan L. Köller 37,371 Grover F. Fuller Jr. 31,780 Jolene W. Appleman 35,428 Karen DeBenecicits 32,977 Kristina L. Konstas 37,864 Lorraine B. Ling 35,251 Seth H. Jacobs 32,140 Garth Butterfield 36,997 Martha A. Gammill 31,820 Garth J. Goddard 39,203 Gregory P. Raymer 36,647 Raymond M. Speer 26,810 E. Victor Donahue 35,492 Jennifer A. Kispert 40,049 Roy F. Waldron 42,208 Israel Nissenbaum 27,582 Todd M. Crissey 37,807 Adrian G. Looney 41,406 Deborah A. Martin 44,222 A. David Joran 37,858 Jeffrey N. Myers 41,213 Lawrence C. Akers 28,587 Elsa Djuardi 45,963 Gabriel L. Kleman 40,681 Michelle A. Sherwood 36,271 Donna R. Grossu P-47,248 Arlene K. Musser 37,895 Arlene K. Musser 37,895 Arlene K. Musser 37,895 Direct all correspondence to:														
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Lawrence C Akers Gabriel L. Kleiman 40,681 40,681 Michelle A. Sherwood 36,271 Michelle A. Sherwood 36,271 Michelle A. Sherwood 36,271 Michelle A. Sherwood 37,895 Arlene K. Musser 37,895 Michelle A. Sherwood 37,895 Michelle A.														
Gabriel L. Kleiman Donna R. Grossu Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to: □Customer Number or Bar Code Label Name Paul H. Ginsburg Address Pfizer Inc Address 235 East 42nd Street, 20th Floor City New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be first, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or impresonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the volidity of the application or any patient issued thereon Name of Sole or First Inventor: □ A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle										S				
Donna R. Grossu P-47,248 Arlene K. Musser 37,895 Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to: ☐ Customer Number or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Correspondence address below or Bar Code Label OR ☐ Code Delay OR ☐ Correspondence address below or Bar Code Label OR ☐ Code Delay OR ☐ Correspondence address below or Bar Code Label OR ☐ Code Label OR ☐ Code Delay OR											- 1			
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Name Paul H. Ginsburg Address Pfizer Inc Address 235 East 42nd Street, 20th Floor City New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made heren of my own knowledge are true and that all statements made on information and belief are believed to be first, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or impresonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeepardize the validity of the application or any patient issued thereon. Name of Sole or First Inventor: A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle					·	Stored 1 ,	ecitioner	monnau	0.1 3.1001	1 10/00/0	20 2012	chec herett		
Address Pfizer Inc Address 235 East 42nd Street, 20th Floor City New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made heren of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or improsoment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon Name of Sole or First Inventor: A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N.	Direct all cone	spondence								OR	⊠ c	orrespond	lence address l	below
Address 235 East 42nd Street, 20th Floor City New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be 1004, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or improsoment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon Name of Sole or First Inventor: A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N.	Name	Paul H. Gin	sburg											
City New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be first, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or improsomment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. Name of Sole or First Inventor: A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle	Address	Pfizer Inc												
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be hot, and jurther that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or improsonment, or both, under 18 USC 1001 and that such willful false statements may jeopardize the validity of the application or any patient issued thereon. Name of Sole or First Inventor: A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle	City	New York			State		New Y	ork_		Zip Co	ode	10017-5	755	
believed to be true, and turther that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or improsoment, or both, under 18 USC 1001 and that such willful false statements may jeepardize the validity of the application or any patent issued thereon. Name of Sole or First Inventor: Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle	Country	United State	es Of America	Te	lephone		(212)5	73-2369		Fax		(212)573	3-1939	
Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle	believed to be punishable by	frus, and fine or imp	turther that thes irisonment, or bo	e statemen	ts were ma	ide with	the kno	owledge	that will	ful false	stateme	ents and I	the like so mad	de are
Harold N. Conkle	Name of Sole	or First In	ventor: A	petition ha	as been file	d for this	s unsign	ed inven	tor				·	
		ven Name	(first and middle	[if any])					Fam	ily Name	or Sur	name		
		γ				Conkl	le							
Signature	·										Date			
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Post Office Address	Post Office A	ddress							************					
Post Office Address 875 Kenridge Court	Post Office A	ddress	875 Kenridge	Court										
City Columbus State OH Zip 43220 Country USA			Columbus	State	ОН	Zip	43:	220	Cou	intry	USA			
Additional inventors are being named on the 3 a supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.	Addition	al inventors	are being name	ed on the 3	a supplem	ental Ac	dditional	Inventor	(s) shee	et(s) PTO	/SB/02	2A attache	ed hereto.	



Name of Additional Joint								
	Inventor, if any	/: D	A petit	ion has bee	en filed for the	s unsigned inver	ntor	
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Inventor's Signature							Date	
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City	Camarillo	State	CA	Zip	93012	Country	USA	
Name of Additional Joint	Inventor, if any	·: 🗆	A petit	ion has bee	en filed for this	s unsigned inver	ntor	
Given Name (fi	irst and middle [i	f any])				Family Name	or Surname	
Scott J.				Blonigen				
Inventor's Signature							Date	
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City	Hilliard	State	ОН	Zip	43026	Country	USA	
Name of Additional Joint	Inventor, if any		A petit	on has bee	n filed for this	s unsigned inver	itor	
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Given Name (fi Fred H. Inventor's Signature Residence: City Post Office Address Post Office Address	Terre Haute  159 Phoenix C Terre Haute	f any]) ourt State	State	Weber IN Zip	<b>Country</b> 47803	Family Name	Date Citizenship	USA
Given Name (fi Fred H. Inventor's Signature Residence: City Post Office Address Post Office Address City Name of Additional Joint	Terre Haute  159 Phoenix C Terre Haute	ourt State	State	Weber IN Zip on has bee	Country 47803 an filed for this	Family Name	Date Citizenship  USA	USA
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Given Name (fi Fred H.  Inventor's Signature Residence: City Post Office Address Post Office Address City  Name of Additional Joint Given Name (fi David R.  Inventor's Signature	Terre Haute  159 Phoenix C  Terre Haute  Inventor, if any rest and middle [inventor]	ourt State	State	Weber IN Zip on has bee	Country 47803 an filed for this	Eamily Name  USA  Country  sunsigned inver	Date Citizenship  USA	USA
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Name of Additional Joint	t Inventor, if any		A petit	ion has bee	en filed for thi	is unsigned inve	ntor	
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Bruce				Monzyk				
Inventor's Signature							Date	
Residence: City	Delaware		State	ОН	Country	USA	Citizenship	USA
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Post Office Address	7460 Blaney R	oad						· · · · · · · · · · · · · · · · · · ·
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Name of Additional Joint	t Inventor, if any	: 🗆	A petit	ion has bee	en filed for thi	s unsigned inve	ntor	
Given Name (f	first and middle [i	f any])				Family Name	e or Surname	
Fimothy M				Werner				<u>-</u>
Inventor's Signature							Date	
Residence: City	Traverse City		State	MI	Country	USA	Citizenship	USA
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Post Office Address	608 W 7th Stre	eet						
City	Traverse City	State	MI	Zip	49684	Country		
Name of Additional Joint	t Inventor, if any	: 0	A petit	ion has bee	en filed for thi	is unsigned inve	ntor	
Given Name (f	first and middle [r	fany])				Family Name	e or Surname	
Chad M.				Cucksey				
Inventor's Signature							Date	
Residence: City	Columbus		State	ОН	Country	USA	Citizenship	USA
Post Office Address			A		L.,	1		<del> </del>
Post Office Address	1704 West 3rd	Avenue						
City	Columbus	State	ОН	Zip	43212	Country	USA	
Name of Additional Joint	t Inventor, if any	: 0	A petit	ion has bee	en filed for the	s unsigned inve	ntor	
Given Name (f	first and middle [i	fany])				Family Name	e or Surname	
Hamish A. I				McArthur				
Inventor's Signature					***************************************		Date	
Residence: City	Mystic		State	CT	Country	USA	Citizenship	USA
Post Office Address								
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City	Mystic	State	СТ	Zip	06355	Country	USA	

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### **DECLARATION**

Name of Additional Joint	Inventor, if any	: [ ]	A petit	on has be	en filed for thi	s unsigned invei	ntor	
Given Name (f	irst and middle [i	fany])				Family Name	or Surname	
Ted L.		_		Tewksbu	ry			
Inventor's	-760	L	10	. Del	J. M.		Date	
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Name of Additional Joint	Inventor, if any		A petit	on has be	en filed for thi	s unsigned inver	ntor	
Given Name (f	irst and middle [i	fany])				Family Name	or Surname	
Inventor's Signature				****			Date	
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	Inventor, if any		A petiti	<u> </u>	en filed for thi	Country s unsigned inver	ntor	
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City  Name of Additional Joint Given Name (f  Inventor's Signature Residence: City Post Office Address		:		<u> </u>		s unsigned inver	or Surname  Date	
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DECLARAT	ION FOR UTILITY OR	Attorney Docket Number	PC10433A
	DESIGN	First Named Inventor	Harold N. Conkle
PATEN	T APPLICATION	COMPLE	TE IF KNOWN
(3)	7 CFR 1.63)	Application Number	09/701,760
Declaration submitted	Declaration Submitted after Initial	Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
with Initial Filing	Filing (surcharge 37 CFR 1 16 (e))	Group Art Unit	Not Yet Assigned
	required)	Examiner Name	Not Yet Assigned

My residence, post office address, and citizenship are as stated below next to my name.  I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:  METHOD FOR THE PURIFICATION, RECOVERY, AND SPORULATION OF CYSTS AND OOCYSTS  The specification of which (Title of the Invention)  Is attached hereto OR was filed on (MM/DD/YYYY) (02/25/2000) as PCT International  Application Number PCT/US00/04733 and was amended on (MM/DD/YYYY)  I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.  I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.  I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate. or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate,
mames are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:  METHOD FOR THE PURIFICATION, RECOVERY, AND SPORULATION OF CYSTS AND OOCYSTS  the specification of which is attached hereto OR was filed on (MM/DD/YYYY) is attached hereto OR Mass filed on (MM/DD/YYYY) is as PCT International  Application Number PCT/US00/04733 and was amended on (MM/DD/YYYY) I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.  I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.  I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate. or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate.
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the specification of which is attached hereto OR was filed on (MM/DD/YYYY) 02/25/2000 as PCT International  Application Number PCT/US00/04733 and was amended on (MM/DD/YYYY)  I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.  I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.  I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate. or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate.
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was filed on (MM/DD/YYYY)  Q2/25/2000  as PCT International  Application Number  PCT/US00/04733  and was amended on (MM/DD/YYYY)  I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.  I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.  I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate. or 365(a) of any PCT international application which designated at least one country other than the United States of America, Isted below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate.
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.  I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.  I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate. or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate.
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.  I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.  I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate. or 365(a) of any PCT international application which designated at least one country other than the United States of America, Isted below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate.
I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate
certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate
or of any PCT international application having a filing date before that of the application on which priority is claimed.
Prior Foreign Application Number(s)  Priority Date Priority Not Claimed YES NO NO Claimed YES NO
Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.
I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below:
Application Number(s) Filing Date (MM/DD/YYYY)  50/122.160 2/26/99

# **DECLARATION ---- Utility or Design Patent Application**

Thereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 U.S.C. 156, which became available between the filling date of the prior application and the national or PCT international filling date of this application.

information which is many and the national or PCT I	atenal to patentabil nternational filing da	ity as defin ite of this app	ed in 37 US/ plication	C 156,	, which became	available	e between	the fil	ling date	of the prior application	
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As a named inventor, I he	ereby appoint the follo	lowing registe	tered practitioner	r(s) to pro	osecute this appl	lication and	d to transa	ct all bu	siness in th	he Patent	
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Name			Registration Number					Registration Number			
Peter C. Richardson	1		27,526		A. Dean					31,185	
Allen J. Spiegel Paul H. Ginsburg			25,749 28,718			E. Brokk		- 1		32,723	
J. Trevor Lumb			28,718 28,567			M. Fedo C. Zielins				33,688	
James T. Jones			28,567 30,561			J. Zielins T. Ronai				34,462 36,257	
Gregg C. Benson		1	30,977			thy Crea				39,156	
Robert F. Sheyka			31,304		Alan L.		190			37,371	
Grover F. Fuller Jr.		1	31,760		Jolene \	W. Apple		- 1		35,428	
Karen DeBenedictis	i	ļ	32,977		Kristina	L. Konst	stas	-		37,864	
Lorraine B. Ling		1	35,251		Seth H.			1		32,140	
Garth Butterfield		1	36,997			A. Gamr				31,820	
Carl J. Goddard		ı	39,203			y P. Rayr		1		36,647	
Raymond M. Speer Jennifer A. Kispert		1	26,810 40,049			r Donahi Waldron				35,492 42,208	
Israel Nissenbaum		l	27,582		Roy F. Waldron Todd M. Crissev				42,208 37,807		
Adrian G. Looney		1	41,406		Deborah A. Martin				44,222		
A. David Joran		1	37,858	Jeffrey N. Myers					41,213		
Lawrence C. Akers		1	28,587	Elsa Djuardi			1		45,963		
Gabriel L. Kleiman		1	40,681		Michelle	e A. Sher				36,271	
Donna R. Grossu			P-47,248			K. Musse				37,895	
Additional register	ed practitioner(s) nar	med on supp	lemental Regist	tered Pra	actitioner Informa	tion sheet	PTO/SB/0	J2C attac	ched heretr	0	
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Name Paul H.	Ginsburg		***************************************								
Address Pfizer In							-				
	t 42nd Street, 20th	ı Floor									
City New Yor	rk		State		New York		Zip Co	ode	10017-5	i755	
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I hereby declare that believed to be true, as punishable by fine or application or any patent	nd further that the Imprisonment or b	ese stateme	ents were made	de with	the knowledge	that willf	lful false :	stateme	e on information	mation and belief are the like so made are	
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	me (first and middl	e (if anyj)				Fami	ily Name	or Surr	name		
Harold N.				Conkle	3						
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Post Office Address											
Post Office Address	875 Kenridg	e Court		,							
City	Columbus	State	e OH	Zip	43220	Cou	ntry	USA			
Additional inven	tors are being nam	ned on the	3 a suppleme	ntal Adr	ditional Invento	or(s) shee	∍t(s) PTO	/SB/02	:A attache	ed hereto.	



Name of Additional Joint	t Inventor, if any	/: D	A petit	ion has be	en filed for thi	s unsigned inver	ntor		
	first and middle [i						or Surname		
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Inventor's Signature					1		Date		
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City	Camarillo	State	CA	Zip	93012	Country	USA		
Name of Additional Joint	Inventor, if any	: 0	A petiti	ion has bee	en filed for this	s unsigned inver	ntor		
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Scott J.				Blonigen					
Inventor's Signature							Date		
Residence: City	Hilliard		State	ОН	Country	USA	Citizenship	USA	
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City	Hilliard	State	ОН	Zip	43026	Country	USA		
Name of Additional Joint	Inventor, if any	: 0	A petiti	on has bee	l en filed for this	s unsigned inver	ntor		
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Name of Additional Joint	l Inventor, if any	/:	A peti	tion has be	en filed for th	is unsigned inver	ntor	
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Given Name (f Chad M. Inventor's	·		A petit		en filed for th	~		
Given Name (f Chad M.	·		A petit		en filed for th	~	or Surname	USA
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Given Name (f Chad M. Inventor's Signature Residence: City Post Office Address Post Office Address City Name of Additional Joint	Columbus  1704 West 3rd	Avenue State	State	Cucksey OH Zip ion has been	Country 43212 en filed for thi	Family Name USA Country	Date Citizenship  USA	USA
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Inventor's Signature							Date	
Residence: City	Columbus		State	ОН	Country	USA	Citizenship	USA
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Inventor's Signature			I				Date	
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Inventor's Signature Residence: City	irst and middle [i	f any])	State		Country	Family Name	Date	
Inventor's Signature Residence: City Post Office Address	irst and middle [t	fany]) State	State	Zip	Country	Family Name	Date	
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Inventor's Signature Residence: City Post Office Address Post Office Address City Name of Additional Joint		State :				Country	Date Citizenship	
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with Initial Filing

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DESIGN

Declaration

required)

Submitted after Initial Filing (surcharge 37 CFR 1.16 (e))

# JOH 7 Registration of use mirouph 9/3APR 2001-51-0032 Patent and Trademark Office U.S. DEPARTMENT OF COMMERCE

Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLE	TE IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet-Assigned

As a below named inventor,	I hereby declare that	t:		41	VBB. SAA.
My residence, post office addr	ess, and citizenship a	re as stated below next to n	nv name.	30	etaff
I believe I am the original, first	and sole inventor (if o	only one name is listed below	w) or an original, first an	d ioint inventor (	endy and the
names are listed below) of the	subject matter which	is claimed and for which a p	atent is sought on the in	nvention entitled:	USTO PIE
As a below named inventor, My residence, post office addr I believe I am the original, first names are listed below) of the METHOD FOR THE PURIFIC	CATION, RECOVERY	, AND SPORULATION OF	CYSTS AND OOCYSTS	: Iura,	
		(Title of the Invention)			
the specification of which		(Title of the liveridor)			
is attached hereto					
was filed on (MM/DD/Y	YYY) 02/25/2000	) as I	PCT International		
Application Number PCT/U	JS00/04733 and v	vas amended on		(if applicab	(ما
	\MMr)	DD/YYYY)			·
I hereby state that I have revise amended by any amendment:			entified specification, in-	cluding the claims	, as
• •			1.6 1: 07.050.4	<b></b>	
i acknowledge the duty to disc	dose information which	is material to patentability	as defined in 37 CFR 1.	56.	
I hereby claim foreign priority to					
certificate, or 365(a) of any Pt America, listed below and hav	e also identified below	, by checking the box, any t	foreign application for pa	atent or inventor's	
or of any PCT international ap	plication having a filing	<del></del>	<u></u>	y is claimed.	
Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Co YES	py Attached? NO
				П	П
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Additional foreign application	numbers are listed or	n a supplemental priority dat	a sheet PTO/SB/02B at	tached hereto.	
hereby claim the benefit unde	er 35 U.S.C 119(e) of	any United States provision	nal application(s) listed to	pelow.	
Application Number(	,	Filing Date (MM/DD/YYYY)			
)/122,160	2/26/99			provisional appli	cation
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## **DECLARATION ---- Utility or Design Patent Application**

Thereby claim the benefit under 35 U S C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and insofar as the subject matter of each of the claims of this application is not disclosed.

information v	vhich is mater	rnational applica ial to patentabili national filing dat	ty as defined	in 37 U.S	ded by C 156	the first i, which l	paragrap became	h of 35 available	USC 1 between	12, I a	icknowledg ling date	e the duty to disc of the prior applica	lose
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Peter C. R Allen J. Sp				27,526			. Dean ervin E		_			31,185	
Paul H. Gi				25,749 28,718			erviii⊏ alerie M					32,723 33,688	
J. Trevor L				28.567			rvan C.					34,462	
James T.				30,561			obert T			Ì		36,257	
Gregg C. E				30,977			. Timoth		igan			39,156	
Robert F.				31,304 31,760			lan L. K					37,371	
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	aren DeBenedictis orraine B. Ling			35,251			eth H. J			i		32,140	
Garth Butt						artha A				31,820			
Carl J. Go			36,997 39,203				Gregory P. Raymer E. Victor Donahue			36,647			
Raymond Jennifer A				26,810 40,049			, victor oy F. W		ue			35,492 42,208	
Israel Niss				27.582			odd M.		,			37,807	
Adrian G. I				41,406			eborah					44,222	
A David J				37,858			effrey N		6	ł		41,213	
Lawrence Gabriel L.				28,587 40,681			lsa Djua ichelle :		avood			45,963	
Donna R.				40,001 P-47,248			rlene K.					36,271 37,895	
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		or	Bar Code La	bel					OK		огтевропе	derice address bei	OW
Name	Paul H Gır	sburg											
Address	Pfizer Inc												
Address	<del></del>	2nd Street, 20th							T				
City	New York	es Of America		State ephone		New Yo	ork 73-2369		Zip Co	ode	10017-5		
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believed to punishable b	be true, and	further that the prisonment, or b	se statements	were mad	de with	the know	wledge	that willf	ul faíse	stateme	ents and	mation and belief the like so made ze the validity of	are
Name of Sc	ole or First In	ventor:	A petition has	been filed	for this	s unsigne	ed inven	tor					
	Given Name	(first and middl	e [if any])					Fami	ly Name	or Sur	name		
Harold N.					Conkl	le							
Inventor's Signature										Date			
Residence:		Columbus		State	Ohio	Cou	untry	USA		Citiz	enship	USA	
Post Office	Address												
Post Office	Address	875 Kenridg	e Court	,									
City		Columbus	State	ОН	Zip	432		Cou		USA			
Additi Additi	onal inventor	s are being nan	ned on the 3 a	suppleme	ental Ac	ditional	Inventor	(s) shee	t(s) PTC	/SB/02	2A attache	ed hereto	

COPOLYNO DHIGH

### **DECLARATION**

Name of Additional Join	Inventor, if any	:	☐ A pet	ition has b	een filed for th	is unsigned inver	ntor		
Given Name (f	irst and middle [i	f any]	)			Family Name	or Surname		
Joseph E.				Schultz					
Inventor's Signature							Date		
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Post Office Address			<del> </del>						_
Post Office Address	11675 Barranc	a Roa	d						
City	Camarillo	Stat	te CA	Zip	93012	Country	USA		
Name of Additional Joint	Inventor, if any	:	☐ A pet	ition has b	een filed for th	ıs unsigned inver	ntor		
Given Name (f	irst and middle [i	f any])	)			Family Name	or Surname		
Scott J.				Blonige	n				
Inventor's Signature			***************************************	1			Date		
Residence; City	Hilliard		State	OH	Country	USA	Citizenship	USA	
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City	Hilliard	Stat	e OH	Zip	43026	Country	USA		
Name of Additional Joint	Inventor, if any	:	☐ A peti	tion has b	een filed for th	is unsigned inver	ntor		
Given Name (f	irst and middle [i	f any])	)			Family Name	or Surname		
Fred H.				Weber					
Inventor's Signature				<del></del>			Date		
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Post Office Address	159 Phoenix C	ourt							
City	Terre Haute	Stat	e IN	Zip	47803	Country	USA		
Name of Additional Joint	Inventor, if any	:	☐ A peti	tion has b	een filed for th	ıs unsıgned inver	ntor		
Given Name (f	irst and middle [i	fany])				Family Name	or Surname		
David R.				Kilanow	ski				
Inventor's Signature							Date		
Residence: City	Dublin		State	ОН	Country	USA	Citizenship	USA	_
Post Office Address						· · · · · · · · · · · · · · · · · · ·		<u> </u>	
Post Office Address	5783 Rushwoo	d Driv	е				·····		
City	Dublin	Stat	e OH	Zin	43017	Country			

# INTERNITOR OF TORA

### **DECLARATION**

Name of Additional Join	it Inventor, if any	/: D	A petit	tion has be	en filed for th	is unsigned inve	ntor	
Given Name (	first and middle [i	f any])				Family Nam	e or Surname	
Bruce				Monzyk				
Inventor's Signature						**************************************	Date	
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City	Delaware	State	ОН	Zip	43015	Country	USA	
Name of Additional Join	t Inventor, if any	/:	A petit	ion has be	en filed for th	is unsigned inve	ntor	
Given Name (	first and middle [i	f any])				Family Nam	e or Surname	
Timothy M.				Werner				
Inventor's Signature						***************************************	Date	
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Post Office Address			.1			L		
Post Office Address	608 W. 7th Str	eet						
City	Traverse City	State	MI	Zip	49684	Country		
Name of Additional Join	t Inventor, if any	: 0	A petit	ion has be	en filed for th	s unsigned inve	ntor	
Given Name (	first and middle [i	f any])				Family Nam	e or Surname	
Chad M.				Cucksey				
Inventor's Signature	Chad	M.	Cu	Mse	uf-		12/29/2000	
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Post Office Address								\
Post Office Address	1704 West 3rd	Avenue						
City	Columbus	State	ОН	Zip	43212	Country	USA	
Name of Additional Join	t Inventor, if any	: 🗆	A petit	ion has be	en filed for thi	s unsigned inve	ntor	
Given Name (	first and middle (i	f any])	İ			Family Nam	e or Surname	
Hamish A. I				McArthur				
Inventor's Signature							Date	
Residence: City	Mystic		State	CT	Country	USA	Citizenship	USA
Post Office Address								
Post Office Address	202 Library Str	eet						<del></del>
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Inventor's Signature	T			L			Date	
Residence. City	Columbus		State	ОН	Country	USA	Citizenship	USA
Post Office Address	1309 Deerlick	Drive						
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City	Columbus	State	e OH	Zip	43228	Country	US	
Name of Additional Joi	nt Inventor, if an	у:	☐ A pet	tion has be	en filed for th	is unsigned inve	entor	
Given Name	(first and middle	[if any])				Family Nam	e or Surname	
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City Name of Additional Joi	nt Inventor, if an	y:		<u> </u>	en filed for th	is unsigned inve	entor ne or Surname	
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Approved for use through 09/30/2000. OMB 0651-0032 Patent and Trademark Office. U.S. DEPARTMENT OF COMMERCE

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DECLARAT	TIÒN FOR UTILITY OR	Attorney Docket Number	PC10433A
-9	DESIGN	First Named Inventor	Harold N. Conkle
PATE	NT APPLICATION	COMPLE	TE IF KNOWN
(3	37 CFR 1.63)	Application Number	09/701,760
Declaration submitted	Declaration Submitted after Initial	Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
with Initial Filing	Filing (surcharge 37 CFR 1.16 (e))	Group Art Unit	Not Yet Assigned
	required)	Examiner Name	Not Yet Assigned

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I believe I am the original, f names are listed below) of	rst and sole invento the subject matter w	r (if only one name is listed below) o hich is claimed and for which a pate	or an original, first an ent is sought on the i	d joint inventor (if nvention entitled:	plural
METHOD FOR THE PURI	FICATION, RECOV	ERY, AND SPORULATION OF CY	STS AND OOCYST	S	
		(Title of the Invention)			
the specification of which is attached hereto					
OR  was filed on (MM/Di	D/YYYY) 02/25	/2000 as PC1	「International		
Application Number PC		and was amended on		(if applicat	ole).
I hereby state that I have re amended by any amendme	viewed and underst	and the contents of the above-ident	ified specification, in	cluding the claims	s, as
I acknowledge the duty to d	isclose information	which is material to patentability as	defined in 37 CFR 1.	56.	
certificate, or 365(a) of any	PCT international a	U.S.C. 119(a)-(d) or 365(b) of any inpplication which designated at leas	t one country other ti	han the United St	
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or of any PCT international  Prior Foreign Application Number(s)	application having a	pelow, by checking the box, any fore filing date before that of the application of the professional parts Foreign Filing Date (MM/DD/YYYY)	eign application for particular priority  Priority  Not Claimed	y is claimed.	opy Attached?
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# COVOLVED DATE

## **DECLARATION ---- Utility or Design Patent Application**

Thereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior

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Peter C. Ri				27,526			A. Dean					31,185	
Allen J. Spi				25,749			Mervin E.					32,723	
Paul H. Gir				28,718			Valerie M			- 1		33,688	
J. Trevor Lu				28,567			Bryan C.			- 1		34,462	
James T. J. Gregg C. B				30,561			Robert T.					36,257	
Robert F. S				30,977 31,304			B. Timoth Alan L. K		gan			39,156	
Grover F. F				31,760			Jolene W		man	- 1		37,371 35,428	
Karen DeB				32,977			Kristina L					37,864	
	rraine B. Ling			35,251			Seth H. J		uo			32,140	
Garth Butte				36.997			Martha A		nill	- 1	32,140 31,820		
Carl J. God	dard			39,203		Gregory P. Raymer					36,647		
Raymond N	<ol> <li>Speer</li> </ol>			26,810 40,049			E. Victor Donahue Roy F. Waldron			35,492 42.208			
Jennifer A.			İ						- 1				
Israel Nisse			27,582		Todd M. Crissey			37.807					
Adrian G. L				41,406			Deborah			- 1		44,222	
A. David Jo Lawrence (				37,858			Jeffrey N		5	- 1		41,213	
Gabriel L. k				28,587 40,681			Elsa Djua Michelle		hoov	1		45,963 36,271	
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Name	Paul H. Gir	nsburg											
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Address	235 East 4	2nd Street, 20th				,					Part		
City	New York			State		<b>—</b>	York		Zip Co	ode	10017-5		
Country	United Stat	es Of America	Tel	ephone		(212	)573-2369		Fax		(212)57	3-1939	
believed to b	e true, and fine or im	statements mad- further that the prisonment, or b and thereon	se statements	s were ma	de with	the k	nowledge 1	that willf	ul false	stateme	ents and	the like so m	nade are
Name of So	le or First Ir	ventor:	A petition has	s been filed	for this	s unsig	ned inven	tor					
	Given Name	(first and middle	e [if any])					Fami	ly Name	or Sun	name		
Harold N.					Conkl	le							
Inventor's Signature										Date			
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Post Office	Address												
Post Office	Address	875 Kenridg	e Court										
City		Columbus	State	ОН	Zip	4	3220	Cou	ntry	USA			
Addition Addition	nal inventor	s are being nam	ed on the 3	a supplem	ental Ad	dition	al Inventor	(s) shee	t(s) PTO	/SB/02	A attache	ed hereto.	

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### **DECLARATION**

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Post Office Address								
Post Office Address	11675 Barrand	a R <b>o</b> ad						
City	Camarillo	State	CA	Zip	93012	Country	USA	
Name of Additional Joi	nt Inventor, if any	(: O	A petit	ion has be	en filed for thi	s unsigned inve	ntor	
Giv∈n Name	(first and middle [i	f any])				Family Name	e or Surname	
Scott J.				Blonigen				
Inventor's Signature							Date	
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Post Office Address	3325 River Na	rrows Roa	d					
City	Hilliard	State	ОН	Zip	43026	Country	USA	
Name of Additional Joi	nt Inventor, if any	/: <u> </u>	A petit	ion has be	en filed for th	s unsigned inve	ntor	
Given Name	(first and middle [i	f any])				Family Name	e or Surname	
Fred H.				Weber				
Inventor's				•	······································		Date	
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Signature Residence: City	Terre Haute		State	IN	Country	ÜSA	Citizenship	USA
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Residence: City	Terre Haute	ourt	State	IN	Country	ÜSA	Citizenship	USA
Residence: City Post Office Address		ourt	State	Zip	47803	USA	Citizenship	USA
Residence: City Post Office Address Post Office Address	159 Phoenix C	State	IN	Zip	47803		USA	USA
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### DECLARATION

Name of Additional Joint	Inventor, if any	: 0	A petiti	on has bee	en filed for this	s unsigned inver	ntor		
Given Name (f	irst and middle (if	fany])				Family Name	or Surname		
Bruce				Monzyk					
Inventor's Signature							Date		
Residence: City	Delaware State			ОН	Country	USA	Citizenship	USA	
Post Office Address		L			1				
Post Office Address	7460 Blaney R								
City	Delaware	State	ОН	Zip	43015	Country	USA		
Name of Additional Joint	Inventor, if any	: 0	A petiti	on has bee	en filed for thi	s unsigned inver	ntor		
Given Name (f	irst and middle [ir	fany])				Family Name	or Surname		
Timothy M.				Werner					
Inventor's Signature	Limoth	n 7	r. Ver	~			Date	12/29/00	
Residence: City	Traverse City		State	MI	Country	USA	Citizenship	USA	
Post Office Address			l	1	l			L	
Post Office Address	608 W. 7th Stre	eet							
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Ted L.				Tewksbu	ry			
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# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

Declaration submitted with Initial Filling

Declaration
Submitted after Initial
Filing (surcharge
37 CFR 1.16 (e))
required)

Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLET	E IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

As a below named inventor, I hereby declare that:								
My residence, post office address, and citizenship are as stated below next to my name.								
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:								
METHOD FOR THE PURIFICATION, RECOVERY, AND SPORULATION OF CYSTS AND OOCYSTS								
the specification of which is attached hereto			(Title of the Invention)	3104				
OR  was filed on (MM/DD)	YYYY) 02/	25/2000	as PCT I	nternational				
	/US00/04733	(MM/E	as amended on DD/YYYY)		(if applicab			
I hereby state that I have revi amended by any amendmen	lewed and under t specifically refe	rstand the erred to	ne contents of the above-identifi above.	ed specification, in	cluding the claims	s, as		
			is material to patentability as d					
certificate, or 365(a) of any F America, listed below and ha	PCT internationa ve also identifie	al applica d below,	C. 119(a)-(d) or 365(b) of any fo ation which designated at least o , by checking the box, any forei , date before that of the applicat	one country other that application for pa	nan the United Statent or inventor's	ates of		
Prior Foreign Application Number(s)	Country		Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Co YES	py Attached? NO		
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☐ Additional foreign applicatio	n numbers are li	isted on	a supplemental priority data sh	eet PTO/SB/02B at	tached hereto:			
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	der 35 U S C 11	19(e) of a						
I hereby claim the benefit und	der 35 U S C 11	19(e) of a	any United States provisional ap	Additional numbers suppleme		sheet		

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## **DECLARATION ---- Utility or Design Patent Application**

I hereby claim the benefit under 35 U S C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of Amenca, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph 35 U S C 112, 1 acknowledge the duty to disclose information which is material to patentiability as defined in 37 U S C 1.56, which became available between the filing date of the prior application and the national or PCT International filing date of this application

information wh	hich is materi	national applicational to patentability rnational filing date	as defined	l in 37 US	C 1.56,	, which	h became :	available	between	the fil	ling date (	of the prior application	on
U.S. Parent	Application Num	Number or PCT nber	Γ Parent		t Filing I/DD/YY				Pa		atent Nur pplicable)		
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Additional U.S. or PCT International application numbers a named inventor, I hereby appoint the following registers.								AND DESCRIPTION OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF THE PARTY OF					
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Allen J. Spi				25,749			Mervin E.			- 1		32,723	-
Paul H. Gin				28,718			Valerie M					33,688	
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Gregg C. B		]		30,977			B. Timoth			- 1		36,257 39,156	
Robert F. S		- 1		31,304			Alan L. Ko		gan	- 1		37,371	
Grover F, F		i		31,760			Jolene W		man	- 1		35,428	
Karen DeBe		- 1		32,977			Kristina L		as			37,864	
Lorraine B.				35,251			Seth H. Ja					32,140	
Garth Butte		1		36,997			Martha A.					31,820	
Carl J. God Raymond N		1		39,203 26,810			Gregory F E. Victor I					36,647 35,492	
Jennifer A.		l		40,049			Roy F. Wa		16			42,208	
Israel Nisse		1		27,582			Todd M. C			- 1		37,807	
Adrian G. L	.ooney	ł		41,406		1	Deborah A	A. Mart	tin	- 1		44,222	
A. David Jo				37,858			Jeffrey N.	. Myers				41,213	
Lawrence C				28,587			Elsa Djua					45,963	
Gabriel L. K				40,681			Michelle A					36,271	
Donna R. G				P-47,248			Arlene K.					37,895	-
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Name	Paul H. Gin	sburg											
Address	Pfizer Inc												
Address	235 East 43	2nd Street, 20th F	Floor										
City	New York			State		New	/ York		Zip Co	ode	10017-5	755	
Country	United State	tes Of America	Tel	ephone		(212)	)573-2369		Fax		(212)573	3-1939	
believed to be	e true, and / fine or imp	further that these prisonment, or bo	e statements	s were mad	de with	the ki	knowledge ti	that willfu	ul false s	stateme	ents and t	rmation and belief at the like so made at ze the validity of tr	ere l
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Harold N.		,			Conkle	е							
Inventor's Signature										Date			
Residence: (		Columbus		State	Ohio	, с	Country	USA		Citiz	enship	USA	
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City		Columbus	State	ОН	Zip	4	13220	Cour	ntry	USA			
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Name of Additional Joint	Inventor, if any	:	A petit	on has been filed for this unsigned inventor				
Given Name (f	irst and middle [i	f any])				Family Name	or Surname	
Joseph E.	$\sim$	$\bigcirc$ 1	Λ,	Senultz				
Inventor's			V				Date	
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Post Office Address	Carlanio		State		Country		Citizenship	USA
Post Office Address	11675 Barranca Road							
City	Camarillo State CA			Zip	93012	Country	USA	
Name of Additional Joint	Inventor, if any	: 📗	A petiti	on has bee	en filed for this	unsigned inver	ntor	
Given Name (f	irst and middle [i	f any])				Family Name	or Surname	
Scott J.				Blonigen				
Inventor's Signature							Date	
Residence: City	Hilliard		State	OH	Country	USA	Citizenship	USA
Post Office Address				J				
Post Office Address	3325 River Nar	rows Roa	d					
City	Hilliard	State	ОН	Zip	43026	Country	USA	
Name of Additional Joint	Inventor, if any	: 0	A petiti	on has bee	en filed for this	unsigned inver	ntor	
Given Name (f	irst and middle [i	f any])		Family Name or Surname				
Fred H.				Weber				
Inventor's Signature							Date	
Residence: City	Terre Haute		State	IN	Country	USA	Citizenship	USA
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Name of Additional Joint	Inventor, if any	: 🗆	A petiti	on has bee	en filed for this	unsigned inver	itor	
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David R.				Kilanowsk	i			
Inventor's Signature							Date	
Residence: City	Dublin		State	ОН	Country	USA	Citizenship	USA
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Post Office Address	5783 Rushwoo	d Drive						
City	Dublin	State	ОН	Zip	43017	Country		



Name of Additional Joint	Inventor, if any	/:	A petit	ion has be	en filed for th	is unsigned inve	ntor	
Given Name (f	first and middle (i	f any])				Family Name	e or Surname	
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Residence: City	Delaware		State	ОН	Country	USA	Citizenship	USA
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Timothy M.				Werner				
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Post Office Address	608 W. 7th Stre	et						
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# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

Declaration submitted with Initial Filing Declaration
Submitted after Initial
Filing (surcharge
37 CFR 1.16 (e))
required)

Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLE	TE IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

As							
	a below named in	ventor, I hereby	declare tha	t:			
Му	residence, post off	ice address, and	citizenship a	re as stated below next to my	name.		
I be nan	lieve I am the origines are listed below	nal, first and sole v) of the subject n	inventor (if on atter which	only one name is listed below is claimed and for which a pa	or an original, first a tent is sought on the	nd joint inventor (i invention entitled.	f plural
ME	ETHOD FOR THE I	PURIFICATION, I	RECOVERY	, AND SPORULATION OF C	YSTS AND OOCYST	'S	
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# **DECLARATION ---- Utility or Design Patent Application**

hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and insofar as the subject matter of each of the claims of this application is not disclosured.

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Gregg C. Benson Robert F. Sheyka				30,977							ļ		
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A. David Jo			-	37,858 Jeffrey			rey N. Myers				41,213		
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Harold N.					Conkl	е			***************************************				
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Name of Additional Joint	Inventor, if any	.   _	A petit	ion has be	en filed for the	s unsigned inver	ntor	
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Joseph E.				Schultz				
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Name of Additional Joint	Inventor, if any	: 0	A petit	ion has bee	en filed for thi	s unsigned inver	ntor	
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Inventor's Signature	Lot	4 B	6:				12/28/90	
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Given Name (f Fred H. Inventor's Signature Residence: City Post Office Address Post Office Address	Terre Haute  159 Phoenix Co	ourt State	State	Weber IN Zip	Country 47803	Family Name	Date Citizenship USA	USA
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Inventor's Signature							Date	
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Timothy M.				Werner				
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### DECLARATION

Name of Additional Join	t Inventor, if any	/: [	☐ A petiti	on has be	en filed for thi	s unsigned inve	ntor	
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# DECLARATION FOR UTILITY OR DESIGN

# PATENT APPLICATION (37 CFR 1.63)

Declaration submitted with Initial Filing

Declaration
 Submitted after Initia
Filing (surcharge
37 CFR 1.16 (e))
required)

Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLETE	IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

My residence, post office address, and citizenship are as stated below next to my name.  I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plura names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:  METHOD FOR THE PURIFICATION, RECOVERY, AND SPORULATION OF CYSTS AND OOCYSTS  (Title of the Invention)	al
names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:  METHOD FOR THE PURIFICATION, RECOVERY, AND SPORULATION OF CYSTS AND OOCYSTS  (Title of the Invention)	al
(Title of the Invention)	
are openioation of which	
s attached hereto	
OR  was filed on (MM/DD/YYYY)  02/25/2000  as PCT International	
Application Number PCT/US00/04733 and was amended on (MM/DD/YYYY) (if applicable).	
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as	
amended by any amendment specifically referred to above.	
I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.	
I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's	s
certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of	of
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America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certific or of any PCT international application having a filing date before that of the application on which priority is claimed.	ficate,
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# **DECLARATION ---- Utility or Design Patent Application**

Thereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 U.S.C. 156, which became available between the filing date of the prior application and the national or PCT International filing date of this application.

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Gregg C. B				30,977				y Crea		- 1		36,257 39,156	
Robert F S				31,304		Alan L. Koller						37,371	
Grover F. F				31,760		Jolene W. Appleman					35,428		
Karen DeBenedictis				32,977				Konst				37,864	
Lorraine B. Ling				35,251			ı H. Ja			- 1		32,140	
Garth Butterfield				36,997				Gamn				31,820	
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Jennifer A.				26,810 40,049				Donahı	16	l		35,492	
Israel Nisse				27,582						42,208 37,807			
Adrian G. L				41,406	Todd M. Crissey Deborah A. Martin					44,222			
A. David Jo				37,858	Jeffrey N. Myers				44,222				
Lawrence C				28,587					45,963				
Gabriel L. K				40,681				. Sher		1		36,271	
Donna R. G				P-47,248				Musse				37,895	
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Name	Paul H Gir	isburg											
Address	Pfizer Inc												
Address		2nd Street, 20th	Floor						Τ				
City	New York			State		New York			Zip Co	ode	10017-5		
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Harold N.					Conk	le							
Inventor's Signature										Date			
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Joseph E.				Schultz					
Inventor's Signature							Date		
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Scott J.				Blonigen					
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Timothy M				Werner				
Inventor's Signature			-				Date	
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Inventor's Signature							Date	
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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

Declaration submitted with Initial Filing Declaration
Submitted after Initial
Filing (surcharge
37 CFR 1.16 (e))
required)

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Attorney Docket Number	PC10433A
First Named Inventor	Harold N. Conkle
COMPLETE	IF KNOWN
Application Number	09/701,760
Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)
Group Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned

As a below named invento	r, I hereby declar	that:			
My residence, post office ad	dress, and citizens	hip are as stated below next to my n	ame.		
I believe I am the original, fin names are listed below) of the	st and sole invento ne subject matter w	r (if only one name is listed below) o hich is claimed and for which a pate	r an original, first ar nt is sought on the i	nd joint inventor (i invention entitled:	f plural
METHOD FOR THE PURIF	ICATION, RECOV	ERY, AND SPORULATION OF CYS	STS AND OOCYST	rs .	
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the specification of which is attached hereto OR		,			
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I hereby state that I have rev amended by any amendmen	iewed and underst t specifically referr	and the contents of the above-identifed to above.	ied specification, in	icluding the claim	s, as
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Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Co	ppy Attached? NO
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I hereby claim the benefit und	der 35 U S C. 119(e) of any United States provisional a	pplication(s) listed t	below:	
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DECLARATION ---- Utility or Design Patent Application

Thereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 385(c) of any PCT international application designating the United States of Amenica, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 U.S.C. 156, which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number or PCT Parent Number (MM/DD/YYY) Additional U.S. or PCT bitarnational application numbers are listed on a supplemental profity data sheet PTO/SBI028 statished neverb as a named inventor. I herethy appoint the following repairmed practitioner(s) to prosecute this application and to transport all counters in the Patent and Taddemark Office opiniorised believes Parent Patent Number Parent Number Parent Number Parent Patent Number Parent	information w	vnich is matei	ernational applicat erial to patentabilit ernational filling date	ity as define	ned in 37 U.S	JC 156	the first parag δ, which beca	raph of me avails	able betwee	112, 1 a	acknowledg	ge the duty to disclose of the prior application
As a named inventor. I hereby appoint the I clowwing registered practicioner(s) to prosecute this application and to transact all business in the Patent and Trademark. Office connected therewith Customer Number and Cross Annumber (s) name/registration number listed below Registration Number N		Application	n Number or PC		Paren				F			
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Name	As a named in	ventor, I herel	by appoint the follo	wing registe	ered practitions	er(s) to p	prosecute this ar	polication	and to trans	sact all bi	isiness in the	he Patent
Name				Custor	mer Number	```F	0	P	M.C.	1		Place Customer
Number Number	L					ner(s) na	me/registration	number li	isted below			
Peter C. Richardson	0.01				Number						—	Number
Paul H. Ginsburg				ſ							-	31,185
J. Trevor Lumb James T. Jones 30,561 Robert T. Romau 36,257 Rogeg C. Benson 30,977 Robert F. Sheyka 31,304 Alan L. Koller 37,371 Jones J. John L. Koller 37,371 John L. Koller 37,371 John L. Konstas 37,864 John L. Koller 37,371 John L. Konstas 37,864 Saren DeBenedictis 32,977 Kristina L. Konstas 37,864 Saren DeBenedictis 32,977 Kristina L. Konstas 37,864 Saren DeBenedictis 36,997 Martha A. Gammill 31,820 Gragony P. Raymer 36,647 Raymond M. Speer 26,810 Saren Martha A. Gammill 31,820 Graymond M. Speer 26,810 Saren Martha A. Gammill 31,820 Graymond M. Speer 26,810 Saren Martha A. Gammill 31,820 Graymond M. Speer 36,647 Raymond M. Speer 26,810 Saren Martha A. Gammill 31,820 Graymond M. Speer 37,858 John Martha A. Gammill 31,820 Graymond M. Speer 37,858 John Martha A. Gammill 31,820 Graymond M. Speer 36,647 Raymond M. Speer 26,810 Saren Martha A. Gammill 31,820 Graymond M. Speer 36,647 Raymond M. Speer 37,858 John Martha A. Gammill 31,820 Graymond M. Speer 36,647 Raymond M. Speer 36,647 Raymond M. Speer 37,858 John Martha A. Gammill 31,820 Graymond M. Speer 36,647 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 37,857 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Speer 38,647 Raymond M. Martin 48,228 Raten M. Martin 48,963 Raymond M. Martin 48,928 Raten M. Martin 48,963 Raymond M. Speer 47,943 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin 48,963 Raymond M. Martin			1	İ						ļ	í	
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Addition G. Looney A. David Joran Lawrence C. Akers A. David Joran Lawrence C. Akers Babel State Bell Kleiman Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto P-47,248 Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to: Customer Number or Bar Code Label Name Paul H Ginsburg Address Pfizer Inc Address Pfizer Inc Address Pfizer Inc Address Pfizer Inc Address Pose Vork State New York Cuntry United States Of America Telephone Direct all satements made herein of my own knowledge are true and that all satements made on information and belief are punishable by fine or imprisonment, or both, under 18 USC 1001 and that such willful false statements made on information and belief are punishable by fine or imprisonment, or both, under 18 USC 1001 and that such willful false statements made the validity of the application or any patent issued thereon Name of Sole or First Inventor: Given Name (first and middle [if any]) A petition has been filed for this unsigned inventor Conkle Post Office Address 875 Kenridge Court City Columbus State OH Zip 43220 Country USA Citizenship USA	Israel Nisse	enbaum	,	l .	27,582		Todd I	M. Criss	sey		1	
A David Joran Lawrence C. Akers Gabriel L. Kleiman A Donna R. Grossu P-47,248 Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to: □ Customer Number or Bar Code Label Name Paul H. Ginsburg Address Pfizer Inc Address Pizer Inc Address 235 East 42nd Street, 20th Floor City New York State New York Vinited States Of America Telephone □ Prescription of further that these statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are purishable by fine or impresoment, or both, under 15 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon Name of Sole or First Inventor: □ A petition has been filed for this unsigned inventor Given Name (first and middle [if any]) A post Office Address 875 Kenridge Court City Columbus State OH Zip 43220 Country USA Jeffice N. Myers Lelsa Djuardi Michalle A. Sherwood Mi			1	į.	41,406		Debor	ah A. M	√artin	- 1		44,222
Gabriel L Kleiman Donna R. Grossu Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to: □Customer Number or Bar Code Label Name Paul H Ginsburg Address Pfizer Inc Address Pizer Inc Address 235 East 42nd Street, 20th Floor City New York State New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or impresoment, or both, under 18 USC 1001 and that such willful false statements may jeopardize the validity of the application or any patient issued thereon Name of Sole or First Inventor: □ A petition has been filled for this unsigned inventor Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle			ļ	í	37,858		Jeffrey	y N. Mye]		41,213
Donne R. Grossu			1	(224	- 1		
Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto Direct all correspondence to:			J	ı						1		
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Name Paul H Ginsburg Address Pfizer Inc Address 235 East 42nd Street, 20th Floor City New York State New York Zip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or impresonment, or both, under 18 U.S.C. 1001 and that such willful false statements may Jeopardize the validity of the apolication or any patient issued thereon Name of Sole or First Inventor: A petition has been filed for this unsigned inventor. Given Name (first and middle [if any]) Family Name or Surname Harold N. Inventor's Signature Residence: City Columbus State Ohio Country USA Citizenship USA Post Office Address 875 Kenridge Court City Columbus State OH Zip 43220 Country USA						tereu - 1.	actitioner mion	iation sile	et P10/56/	02C attac	ched neres	3
Address 235 East 42nd Street, 20th Floor City New York State New York 2ip Code 10017-5755 Country United States Of America Telephone (212)573-2369 Fax (212)573-1939 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such willful false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such will false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such will false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such will false statements and the like so made are punishable by fine or impresonment, or both, unter 18 U.S.C. 1001 and that such will false statements and the like so made are true and that all statements and the like so made are punishable by fine or impresonment, or formation and belief are the statements and that such will false statements and the like so made are punishable by fine or impresonment, or for formation and that all statements and the like so made are punishable by fine	Direct an oc	еѕринчени							OR	⊠ c	orrespond	lence address below
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are purishable by fine or imprisonment, or both, under 18 USC 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. Name of Sole or First Inventor: Given Name (first and middle [if any]) Family Name or Surname Harold N. Conkle Inventor's Signature Residence: City Columbus State Ohio Country USA Citizenship USA Post Office Address Post Office Address 875 Kenridge Court City Columbus State OH Zip 43220 Country USA									Zip C	Code	10017-5	755
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Harold N. Conkle					as been filed	I for this	s unsigned in	entor				
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Additional inventors are being named on the 3 a supplemental Additional inventor(s) sheet(s) PTO/SB/02A attached hereto.												
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Given Name (f	first and middle [i	if any])				Family Name		
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Inventor's Signature							Date	
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Timothy M					Werner						
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Chad M.				-	Cucksey						
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Hamish A. I.					McArthur						
Inventor's Signature					****			Date	· · · · · · · · · · · · · · · · · · ·		
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DECLARATI	ON FOR UTILITY OR	Attorney Docket Number	PC10433A		
	DESIGN	First Named Inventor	Harold N. Conkle		
PATEN [*]	T APPLICATION	COMPL	ETE IF KNOWN		
(37 CFR 1.63)		Application Number	09/701,760		
Declaration Declaration Submitted Submitted Submitted		Filing Date	November 30, 2000 (USA) February 25, 2000 (PCT)		
with Initial Filing (surcharge Filing 37 CFR 1.16 (e))		Group Art Unit	Not Yet Assigned		
required)	Examiner Name	Not Yet Assigned			

As a below named inventor	, I hereby declare the	at:			
My residence, post office add	dress, and citizenship	are as stated below next to my na	ame.		
I believe I am the original, firs	st and sole inventor (if	only one name is listed below) on is claimed and for which a pater	an original, first ar	id joint inventor (i nvention entitled:	f plural
METHOD FOR THE PURIF	ICATION, RECOVER'	Y, AND SPORULATION OF CYS	TS AND OOCYST	S	
the specification of which is attached hereto		(Title of the Invention)			
OR was filed on (MM/DD)	YYYY) 02/25/200	00 as PCT	International		
Application Number PCT		was amended on I/DD/YYYY)		(if applicat	ole)
I hereby state that I have revi amended by any amendment	ewed and understand	the contents of the above-identif	ied specification, in	cluding the claim	s, as
		ch is material to patentability as d			
America, listed below and ha	CT international appli ve also identified belov	s.C. 119(a)-(d) or 365(b) of any focation which designated at least w, by checking the box, any foreign date before that of the applicat	one country other to an application for n	nan the United St	ates of
Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Co	ppy Attached? NO
Additional foreign application	numbers are listed o	n a supplemental priority data sh	eet PTO/SB/02B at	tached hereto:	
		f any United States provisional a			
Application Number		Filing Date (MM/DD/YYYY)			
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Paul H. Gir J. Trevor L				28,718 28,567				. Fedov Zielinsk				33,688	
James T. J				30,561				Ronau				34,462 36,257	
Gregg C. E				30,977				y Crea				39,156	
Robert F. S				31,304		Alan	L. Ko	oller	•			37,371	
Grover F. F				31,760				. Apple				35,428	
Karen DeB				32,977				. Konst	as	1		37,864	
Lorraine B. Garth Butte				35,251 36,997	Seth H. Jacobs Martha A. Gammill			nill	32,140 31,820				
Carl J. Goo				39,203				P. Rayn			36,647		
Raymond N	V. Speer			26,810				Donahi				35,492	
Jennifer A.			ĺ	40,049		Roy	F. Wa	aldron				42,208	
Israel Nisse				27,582				Crissey		- 1		37,807	
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Gabriel L. I			Ì	40.681				اما ۱. Sher	hoow			36,271	
Donna R. C	Brossu			P-47,248				Musse				37,895	
Additio	nal registered	practitioner(s) nar	ned on supple	mental Regis	tered Pra	actitioner Info	rmatio	n sheet F	TO/SB/	02C atta	ched heret	lo.	
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		or	Bar Code La	abel					Oit	2	orrespon	derice address	, Delow
Name	Paul H. Gir	nsburg											
Address	Pfizer Inc												
Address	235 East 4	2nd Street, 20th	Floor										
City	New York			State		New York			Zip C	ode	10017-	5755	
Country	United Stat	tes Of America	Tel	ephone		(212)573-	2369		Fax		(212)57	'3-1939	
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Name of So	le or First Ir	ventor:	A petition ha	s been filed	for this	unsigned i	nvento	or					
	Given Name	(first and middl	e [if any])					Famil	y Name	or Sur	name		
Harold N.					Conkle	е							······································
Inventor's Signature										Date	!		
Residence:	City	Columbus		State	Ohio	Count	ry I	USA		Citiz	enship	USA	
Post Office					1 20	1 2711				1		1 30/1	
Post Office	Address	875 Kenridg	e Court	*****									
City		Columbus	State	ОН	Zip	43220		Cour	ntry	USA			
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Additional inventors are being named on the 3 a supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

CAFATATA CHASCA

DECLARATION

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Name of Additional Join	· · · · · · · · · · · · · · · · · · ·		A petit	ion has be	en filed for thi	is unsigned inve		
	first and middle [if any])		Schultz		Family Nam	e or Surname	
Joseph E.				OCHOIZ				
Inventor's Signature							Date	
Residence: City	Camarillo		State	CA	Country	USA	Citizenship	USA
Post Office Address			-/					
Post Office Address	11675 Barrano	a Road						
City	Camarillo	State	CA	Zip	93012	Country	USA	
Name of Additional Join	t Inventor, if any	/:	A petit	on has be	en filed for thi	is unsigned inve	ntor	
Given Name (first and middle [i	if any])				Family Name	e or Surname	
Scott J.				Blonigen				
Inventor's Signature							Date	
Residence: City	Hilliard		State	ОН	Country	USA	Citizenship	USA
Post Office Address					.1			
Post Office Address	3325 River Na	rrows Roa	id					
City	Hilliard	State	ОН	Zip	43026	Country	USA	
Name of Additional Join	t Inventor, if any	r: 🗆	A petit	ion has be	en filed for thi	s unsigned inve	ntor	
Given Name (first and middle [i	f any])				Family Name	e or Sumame	
Fred H.				Weber				
Inventor's Signature							Date	
Residence: City	Terre Haute		State	IN	Country	USA	Citizenship	USA
Post Office Address								
Post Office Address	159 Phoenix C	ourt						
City	Terre Haute	State	IN	Zip	47803	Country	USA	
Name of Additional Joint	lnventor, if any	: 🗆	A petiti	on has bee	en filed for thi	s unsigned inve	ntor	
Given Name (f	first and middle [i	fany])				Family Name	or Surname	
David R.				Kilanowsk	d			
Inventor's Signature							Date	
Residence: City	Dublin		State	OH	Country	USA	Citizenship	USA
Post Office Address			4					
Post Office Address	5783 Rushwoo	d Drive						
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Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor									
Given Name (first and middle [if any])				Family Name or Surname					
Bruce			Monzyk						
Inventor's	1 K	1 4	7	12/	7		Date		
Signature Residence: City	Delaware		State	OH Country USA		USA	12/30/2000 Citizenship	USA	
Post Office Address		P					L		
Post Office Address	7460 Blaney Road								
					T				
City	Delaware	State	ОН	Zip	43015	Country	USA		
Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor									
Given Name (f	Given Name (first and middle [if any])				Family Name or Surname				
Timothy M.	Werner								
Inventor's Signature						· · · · · · · · · · · · · · · · · · ·	Date		
Residence: City	Traverse City	State	MI	Country	USA	Citizenship	USA		
Post Office Address								I	
Post Office Address	608 W 7th Street								
City	Traverse City	State	MI	Zip	49684	Country			
Name of Additional Joint	Inventor, if any	: 🗆	A petit	ion has bee	en filed for thi	s unsigned inver	ntor		
Given Name (f	irst and middle [i	f any])		Family Name or Surname					
Chad M.				Cucksey					
Inventor's							Date		
Signature Residence: City	Columbus		State	ТОН	Country	USA	Citizenship	USA	
Post Office Address	State 5tate				Country		Ottizenstrip	00/1	
Post Office Address	1704 West 3rd	Avronuo							
City	Columbus	State	ОН	Zip	43212	Country	USA		
Name of Additional Joint	Inventor, if any	: 0	A petit	ion has bee	en filed for thi	s unsigned inver	ntor		
Given Name (first and middle [if any])				Family Name or Surname					
Hamish A. I.				McArthur					
Inventor's Signature						Date			
Residence: City	Mystic		State	CT	Country	USA	Citizenship	USA	
Post Office Address				1	L			· · · · · · · · · · · · · · · · · · ·	
Post Office Address	202 Library Street								
City	Mystic	State	СТ	Zip	06355	Country	USA		

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Ted L. Tew Name (first and middle [if any])	Given Name (fil Ted L. Inventor's Signature Residence: City			A penn	OII IIas per	en nieu ioi un	s unsigned inver	1101			
Tevel L. Inventor's Signature Residence: City Columbus State OH Country USA Citizenship USA Post Office Address City Columbus State OH Zip 43228 Country US Name of Additional Joint Inventor, if any: City State OH Zip 43228 Country US A petition has been filed for this unsigned inventor Family Name or Surname City State Country Citizenship Date Signature Residence: City State Country Citizenship Name of Additional Joint Inventor, if any: State Country Country Date Country Citizenship Date Country State Country Country Date Country State Country Date Signature Country State Country Date Country Date Country Date Country Date Country Date Country Country Country Date Country Date Country Date Country Date Country Country Date Country Date Country Date Country Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname Family Name or Surname	Ted L. Inventor's Signature Residence: City	ist and middle (i	ally])		tion has been filed for this unsigned inventor						
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